Potential *Prunus* Host Range of PPV-PENN Isolates by Aphid Transmission

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Abstract

Natural spread of *Plum pox potyvirus* (PPV) occurs by aphid transmission, grafting, or movement of infected nursery stock. Two proven aphid vectors of *Plum pox virus*, *Myzus persicae* and/or *Brachycaudus persicae* were used to transmit North American isolates of PPV to a range of commercial and ornamental *Prunus* species to test the ability of these species to function as PPV reservoirs. Inoculum sources were 'Lovell' peach seedlings infected with the PPV-PENN-3 or PPV-PENN-4 isolates by previous aphid transmission. Aphids were starved for 30 min, placed onto either detached symptomatic PPV-infected peach leaves or intact peach seedlings, and then allowed a 3-day acquisition-inoculation feeding period from infected tissues to the healthy *Prunus* seedlings. Seedlings were sprayed with insecticide, placed onto greenhouse benches and observed for 6 to 8 weeks for development of symptoms. Test plants were analyzed by ELISA and PCR, and then back-assayed to 'Lovell' peach seedlings by healthy *Myzus persicae*. Test plants then were vernalized at 4.1° C for 8-10 weeks. At four-weeks post-vernalization, plants were tested by ELISA and/or RT-PCR to verify systemic infection. Fourteen of the 15 *Prunus* species tested positive for PPV infection following the initial aphid transmission. Twelve of the 14 infected species were verified to function as potential PPV sources in subsequent aphid transmissions to peach. To date, at least 10 of the 14 *Prunus* species maintained systemic PPV infection following vernalization. Only *P. cerasus* (sour cherry) tested negative to PPV infection. Other cherry species, including *P. avium*, *P. cistena*, and *P. serrulata* maintained systemic infection through vernalization. Results suggest that all species tested except *P. cerasus*, could function as PPV hosts associated with unintentional movement of infected nursery stock and as reservoirs for aphid transmission.

INTRODUCTION

Plum pox (sharka) is the most important virus disease of *Prunus* worldwide and is caused by *Plum pox virus* (PPV: Genus *Potyvirus*; Family *Potyviridae*) (Nemeth, 1986). It was identified in the USA in 1999 (Levy et al., 2000) and has been treated as an eradicable disease since that date. Plum pox is spread short distances among trees within and among orchards by several aphid species (Labonne et al., 1995). This form of spread is controlled by removal of all commercial *Prunus* orchards and homeowner *Prunus* trees within 500 meters of the nearest infected tree. These control measures have reduced the occurrence of new, identifiable positive trees each year since 2000. However, this does not preclude the possibility of aphids moving the virus to wild or ornamental *Prunus* in the vicinity of infected commercial trees. Although there have been no positive identifications among wild species in the quarantine area of Pennsylvania (J. Halbrendt, Pennsylvania State University, personal communication), there have been infected homeowner ornamental plantings (D. Albright, USDA- APHIS, personal communication). Several wild *Prunus* species have been listed as susceptible to PPV (Jordovic, 1965; Nemeth, 1986; Polak, 1997, 1999, 2001).

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Studies were initiated in 2000 in the USA to study the possible role of wild and ornamental *Prunus* species in the establishment and epidemiology of PPV. We report here the preliminary results of studies utilizing seedlings started from healthy seed and aphid transmission of two PPV isolates.

MATERIALS AND METHODS

Several aphid species have been evaluated as potential vectors of PPV isolates in Pennsylvania (Gildow et al., 2004) and from those species *Myzus persicae* (green peach aphid) was chosen for most host range transmission experiments. The black peach aphid (*Brachycaudus persicae*) was used in a few transmission experiments.

Seeds of *Prunus* species and seedlings of one species (*P. glandulosa)* were obtained from commercial seed sources (Table 1). Considerable difficulty was encountered in germination of several wild species. Several stratification regimes of alternating chilling and warming, repeated germination trials, and information obtained from a treatise by Grisez et al., were used to produce healthy test seedlings. A goal of at least 10 healthy seedlings per species was accomplished in most cases, although a few species had fewer seedlings. Continuing germination experiments are in progress for the species with low numbers (Table 1) and several additional species are currently being tested.

Seedlings of different species were transplanted into a potting mix (MetroMix 510 [Scotts Sierra Horticultural Products Co., Marysville, USA]) when cotyledons were fully expanded. Inoculations with PPV were conducted in the 4-8 leaf stage by one of two methods: (1) unlimited healthy aphids were placed on detached leaves of PPV-infected 'Lovell' peaches (Isolates PPV-PENN 3 (Cumberland County) and PPV-PENN 4 (Franklin County)), allowed to roam and probe for at least 30 minutes, and then placed on individual test seedlings in small tubular cages for an inoculation feeding period of 48 hrs; (2) healthy aphids were placed on an infected, intact Lovell peach seedling, allowed to settle on the plant and then the plant was placed in a large cage with small test seedlings and aphids were allowed to move to the seedlings at their volition. Both methods employed a version of Afree roaming@ where aphids were allowed to move from infected leaves to test seedlings and return. Following the inoculation test period, seedlings were sprayed with insecticide and placed on glasshouse benches for 30-90 days observation.

Symptoms were recorded as they appeared on inoculated leaves and newly expanding leaves. Young, fully expanded leaves of all seedlings were analyzed by ELISA utilizing the Durviz-REAL (Valencia, Spain) PPV kit. Absorbance values exceeding 4 times the standard deviation of healthy controls was considered positive. Seedlings also were analyzed by real-time, fluorescent, reverse transcription-polymerase chain reaction (RT-PCR) or by conventional RT-PCR assay. Results were considered positive for real-time, RT-PCR when the reporter fluorescence exceeded the background and for conventional RT-PCR when the product of expected size was amplified (Schneider et al., 2004). Healthy *M. persicae* were used to attempt back inoculations of PPV from the symptomatic, ELISA positive, or PCR positive test plants back to healthy Lovell peach seedlings to complete Koch postulates.

Following analyses and back inoculations, the leaves were stripped from the test seedlings before vernalization in a coldroom at 4.1° C for 8 weeks. After vernalization, the seedlings were allowed to resume growth and observed for symptoms. Any asymptomatic and a random sample of those seedlings with symptoms was re-evaluated by ELISA to confirm persistent systemic infection.

RESULTS AND DISCUSSION

Isolates of PPV from Pennsylvania, USA were able to systemically infect 14 of 15 *Prunus* species inoculated by aphids and only sour cherry (*P. cerasus*) was not infected. Seedlings were observed for symptoms from 14 days post inoculation (PI) until at least 60 days. Symptoms were striking in some species (*P. cerasifera, P. domestica, P. mume,* and *P.*

persica) with chlorotic rings, veinal associated patterns, leaf deformation, and stunting. In other species (*P. salicina,* and *P. serrulata*) symptoms were transient, mild, and more difficult to observe. In all cases, known susceptible *P. persica* cvs GF 305 and Lovell seedlings exhibited strong chlorotic vein patterns, leaf curling and distortion.

Several wild and ornamental *Prunus* species have been described as susceptible or resistant (even immune) to PPV in Europe (Nemeth, 1986) including *P. emarginata, P. padus, P. serotina, P. virginiana, P. yedoensis, P. cerasus,* and *P. avium.* In many cases these reports did not distinguish the strain being tested. Using two isolates of PPV-PENN (strain D) from Pennsylvania, we detected systemic, persistent infection in 14 *Prunus* species (Table 1). Both almond cultivars (*P. amygdalus*), were systemically infected although symptoms were mild and transient (Table 1). Representative seedlings of dwarf flowering almond (*P. glandulosa*) and flowering almond (*P. triloba*) developed systemic chlorotic patterns on new leaves and were positive by ELISA and PCR (Table 1). Sand cherry (*P. cistena*) produced very mild symptoms, was very weak in ELISA and PCR, and back-assays to peach were negative (Table 1). Sweet and tart (sour) cherries have been reported to be infected by PPV-D and other PPV strains but only PPV-C is known to persist systemically in cherries (Nemchinov et al, 1998). We were not able to infect *P. cerasus* (sour cherry) as evidenced by ELISA and PCR results although seedlings produced strong virus-like symptoms including stunting, distorted leaves, and mosaic patterns. On the other hand, *P. avium* (sweet cherry) did not produce clearly discernible symptoms but systemic virus infection was detectable by ELISA, PCR, and back-assay back to peach both before and after vernalization indicating that the virus was systemic in the young plants and did persist through dormancy. However, these results were with young seedlings under ideal disease conditions and we have not carried the cherry plants beyond the first vernalization.

The PPV-PENN isolates have been characterized as PPV-D, however, aphid transmission rates at high levels from peach to peach (Gildow et al., 2004), and what appears to be a unique host range difference within the cherry sub-genus, indicates that U.S. isolates of PPV are biologically different from most reported PPV-D isolates from peach in Europe.

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Tables

Table 1. List of *Prunus* species tested as potential hosts of U.S. isolates of *Plum pox virus* (PPV)

Genus species	Common name	Visual Symptoms	ELISA	PCR	Back Assay	Post Vernalization	Seed Source
Prunus amygdalus	Butte & Mission almond	3/20	+	+	+	+	2
P. armeniaca	Apricot	12/19	+	+	+	+	1
P. avium	Mazzard (sweet) cherry	25/58	+	+	+	+	1
P. cerasifera	Myrobalan plum	12/14	+	+	+	+	1
P. cerasus	Sour (tart) cherry	0/12	-	-	P	P	1
P. cistena	Sand cherry	10/65	+	+	-	+	1
P. domestica	Garden plum	4/4	+	+	+	+	3
P. insititia	Bullace plum	1/2	+	P	+	+	1
P. glandulosa	Dwarf flowering almond	2/2	+	+	NA	-	4
P. mahaleb	Mahaleb cherry	14/74	+	P	+	+	1
P. mume	Japanese apricot	12/12	+	+	+	+	1
P. persica cultivars	Peach	many	+	+	+	+	
P. salicina	Japanese plum	1/6	+	+	+	P	1
P. serrulata	Oriental flowering cherry	17/46	+	+	+	+	1
P. triloba	Flowering almond	2/4	+	+	+	P	1

ELISA + = 4x standard deviation of healthy controls
Real-time RT-PCR + = Reporter fluorescence greater than background
RT-PCR + = amplification of expected product size
- = negative
P = pending
NA = not attempted

Seed Sources

1 = Lawyers Nursery, Inc., Plains, MT
2 = Wilson's Nursery, Hickman, CA
3 = Ralph Scorza, AFRS, Kearneysville, WV
4 = Lowe's Garden Center, Frederick, MD